PHENOTYPIC DIFFERENCES BETWEEN AMERICAN AND SOUTHEAST ASIAN STRAINS OF DENGUE SEROTYPE 2 VIRUSES

Thaisomboonsuk BK, Watts DM, Mammen MP Jr and Endy TP

Dengue virus continues to be a global health concern with considerable morbidity and mortality in the tropical and subtropical regions of the world. Previous studies have demon-strated that American strains of dengue serotype 2 (D2) viruses are genetically different from Southeast Asian strains and infection results in at most mild dengue disease without hemorrhagic manifestations or shock. Previous studies have reported that heparan sulfate is a specific receptor for dengue virus. Heparin has been demonstrated to competitively inhibit dengue virus infection though this effect varies by the dengue serotype suggesting differences in heparan sulfate avidity and possible alternate receptor pathways for viral entry. In our study, the interaction of D2 virus (Asian and American strains) with its cellular receptor and inhibition of virus binding by heparin was determined and compared using cell binding assay with H^3-labeled D2(NGC) and D2 American (Peru) virus strains. Binding activities of 3H5, 4G2 and 2H2 monoclonal antibodies against both strains were determined by enzymeimmunoassay (EIA). Our results demonstrated that heparin inhibition of virus binding and endpoint binding reactivity of 3H5, (serotype-specific neutralizing activity) and 4G2 (flavivirus group determinant) to D2 (Peru) and D2 (NGC) are significantly different between the Asian and American D2 virus strains indicating phenotypic differences between these two viruses. Competitive infection assays between heparin and monoclonal antibodies showed that in D2 (Peru), heparin inhibited binding of 3H5 while in D2 (NGC), binding to both sites was promoted. Our results suggest that the heparan sulfate-binding site and 3H5 neutralizing epitopes are different for the two strains and are adjacent to or overlap with each other. Understanding the phenotypic differences in these two viruses will increase our understanding of the differences in virus entry and infectivity of these viruses and their role in producing subclinical to severe dengue disease.

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POSSIBLE RELATIONSHIP BETWEEN IMMUNE RESPONSE TO MOSQUITO (*AEDES AEGYPTI*) SALIVARY PROTEINS AND DENGUE DISEASE SEVERITY

Machain-Williams C, Troyer J, Mammen, MP Jr, Beaty BJ, Nisalak A and Blair CD

Dengue viruses are arthropod-borne viruses transmitted by Aedes aegypti mosquitoes. These viruses can cause dengue fever (DF), which is a relatively benign disease, or the more severe dengue hemorrhagic fever (DHF) in humans. It is known that arthropod saliva contains proteins that can interfere with the host immune response as well as the coagulation cascade to facilitate the acquisition of blood during the intake of the blood meal. Salivary proteins have usually been characterized after extraction from dissected salivary glands. We developed a procedure

to collect mosquito saliva, directly concentrate proteins by trichloroacetic acid precipitation, and fractionate them by non-denaturing PAGE. We performed immunoblot analysis with these proteins and sera from 200 Thai children who had been diagnosed with DF, DHF, or no dengue virus infection. We showed a possible correlation between the presence of antibodies to certain Aedes aegypti saliva proteins and severity of disease. These results suggest that the immune response to vector mosquito salivary proteins might play a role in the outcome of this arboviral disease.

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PROBLEMS ENCOUNTERED IN THE MOLECULAR DETECTION OF DENGUE VIRUSES

Zhang C

The dengue virus (DENV) molecular typing method, reverse transcriptase - polymerase chain reaction (RT-PCR), described by Lanciotti (Lanciotti et al., 1992) has been used in many laboratories for detecting DENV infection. However, we currently have observed some non-specific results when using this method to detect DENV infection. Some samples showed evidence for multiple infections by two different dengue serotypes, in most cases, DENV-1 plus another serotype. Additionally, some samples gave positive results for DENV-1 by serological tests or show a right size of DNA fragment amplified from the first-round PCR, but negative results by the nested PCR (the second-round) in agarose gel. These non-specific results thus interfered with our ability to accurately detect DENV infection by using this technique. We designed a few of new primers based on our Thai sequence database of DENV variants to modify Lanciotti's RT-PCR and eliminate non-specific reactions in detecting Thai DENV variants.

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A RANDOMIZED, PLACEBO-CONTROLLED, STUDY OF NON-PEGYLATED AND PEGYLATED FORMS OF RECOMBINANT HUMAN INTERFERON-α-2A FOR SUPPRESSION OF DENGUE VIREMIA IN RHESUS MACAQUES

Ajariyakhajorn C, Mammen MP, Endy TP, Gettayacamin M, Nisalak A, Nimmannitya S and Libraty DH

Infection with any dengue viruses can produce a spectrum of disease, ranging from a mild febrile illness, to classic dengue fever, to the most severe form, dengue hemorrhagic fever (DHF). There is growing evidence that viremia levels and the overall viral burden are greatest in DHF. A therapeutic intervention to suppress viremia early in dengue infection could potentially ameliorate severe disease. Two sequential studies examined the effects of recombinant interferon-α (rIFN-α)-2a (Roferon®-A) and pegylated rIFN-α-2a (PEGASYS®) on dengue-2 (D2) viremia